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ULTRASTRUCTURE OF MALE GENITAL SYSTEM AND SPERMATOZOA OF A MEXICAN CAMEL-SPIDER OF THE *EREMOBATES PALLIPES* SPECIES GROUP (ARACHNIDA, SOLIFUGAE)

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ABSTRACT. The male genital system of Solifugae is divided into three different parts: a) a common genital chamber, b) the paired tubular vasa deferentia and c) the long, thin testes. On each side, the vas deferens splits into two smaller branches resulting in the thin, extremely long testes such that one individual possesses four tubular testes in total. The epithelium of a testis consists mainly of a glandular part and of a germinal part surrounded by a small layer of muscles. In *Eremobates* sp., within the germinal part the sperm cells are groups of a few, probably four, mature sperm cells each surrounded by thin extensions of somatic cells. These somatic cells can clearly be distinguished from the cells forming the glandular part which contain large amounts of rough endoplasmic reticulum. Once released into the narrow testicular lumen, the spermatozoa float more or less individually in a proteinaceous secretion. Earlier stages of spermatogenesis could not be detected, suggesting that spermatogenesis may occur in the subadult male (not examined in this study). In general, the sperm is rather simple, representing a round or slightly elongated cell devoid of a flagellum. The relatively small and flat acrosomal vacuole is attached to the disc-like nucleus. The acrosomal filament penetrates the nucleus and is coiled several times around it. In contrast to species of the family Ammotrechidae or Karschiidae, for which sperm cells have already been described, the sperm cells of the Mexican *Eremobates* sp., which belongs to the family Eremobatidae, show no tendency to form any piles or well ordered groups in the lumen of either the testes or the vasa deferentia.

Keywords: Solifugae, genital system, sperm cell, systematics

Most camel-spiders (Arachnida, Solifugae), also called sunspiders or wind-scorpions, inhabit tropical, subtropical regions and arid environments in southern Europe, Africa, Asia and the Americas (Punzo 1998). The oldest specimen of Solifugae is known from the Upper Carboniferous (Pennsylvanian in US terminology) of Mazon Creek, Illinois, USA (Selden & Shear 1996). Most of the 1084 recent species (Harvey 2002) are nocturnal predators known for their extreme rapidity. The huge chelicerae represent a characteristic feature of their external morphology and they can be easily distinguished from other arachnids by the presence of racquet organs (malleoli). Their position within the Arachnida is not yet fully resolved, since Solifugae express both apomorphic (e. g. highly developed tracheal system, two-jointed chelicerae) and plesiomorphic (e. g. segmentation of the opisthosoma) characteristics (Roewer 1934; Moritz 1993), but they are usually considered to be the sister–group of the Pseudoscorpiones (Weygoldt & Paulus 1979; Shultz 1990; Weygoldt 1998; Wheeler & Hayashi 1998; Dunlop 2000; Giribet et al. 2002). In any case, Roewers classification of the order Solifugae is based on a small set of character systems and therefore lacks a reliable basis for phylogenetic and subsequent systematic implications (Harvey 2002).

So far, only a few electron microscopic studies on this animal group have been completed (see e.g., Brownell & Farley 1974; Alberti 1979, 1980; Bauchhenss 1983; Ludwig & Alberti 1992; Alberti & Peretti 2002). Ac-

cording to the current literature, the ventrally located male genital system of Solifugae is generally divided into three different parts: a) a common genital chamber, b) the paired tubular vasa deferentia and c) the long, thin testes. Even though there are several studies on this organ system (see e.g. Roewer 1934; Warren 1939; Junqua 1966), the nomenclature concerning the different parts of the genital system varies considerably between these authors. Only the testes and partly the vasa deferentia have been fine-structurally investigated (Alberti 1980; Alberti & Peretti 2002). The aim of the present study was to confirm and to substantiate the present knowledge on the male reproductive system and sperm morphology and to present the first ultrastructural study of the genital chamber and its accessory glands.

METHODS

Males of the genus *Eremobates* Banks 1900, belonging to the *Eremobates pallipes* (Say 1823) species group according to Brookhart (pers. comm.), were captured near Pachuca-City, State of Hidalgo, Mexico (20°07′21″N, 98°44′09″W). After dissection of three males in ice-cold cacodylate buffer their genital systems were fixed in 3.5 % glutaraldehyde buffered in cacodylate buffer (pH 7.4; 0.1 M). Fixed genital systems were sent to Germany in diluted glutaraldehyde. Postfixation processes included treatment with OsO_4 (2 %) for two hours, rinsing in buffer solutions, dehydration in graded ethanols (60–100 %) and embedding in Spurrs medium (Spurr 1969). Ultrathin sections of approximately 70 nm were cut with a Diatome diamond knife using a Leica Ultracut microtome. Sections were stained with saturated uranylacetate (in 70 % methanol) and lead citrate according to Reynolds (1963). For general orientation semithin sections (700 nm) were used which were stained according to the methods of Richardson et al. (1960). Transmission electron microscopy was performed using a Zeiss EM 10 A transmission electron microscope. For scanning electron microscopy, the genital system was dehydrated in graded ethanols (60–100 %), then coated with gold-palladium and finally investigated with a LEO DSM 940. A male *Eremobates* sp. has been deposited as a voucher specimen in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN) in Buenos Aires.

RESULTS

Scanning electron microscopical observations.—In general, the male genital system consists of a common genital chamber, the vasa deferentia and the testes. Immediately after being removed from the male, the fresh genital system is translucent yellow. The paired tubular vasa deferentia originate from the genital chamber to which small accessory glands are directly attached. Each vas deferens splits into two smaller branches each resulting in extremely long, thin testes which are only partly shown in Fig. 1.

Light and transmission electron microscopical observations.—*Testes:* The long, thin tubular testes are surrounded by small muscle cells. The somatic epithelium is composed of a larger glandular and a comparatively small part in which the germinal cells are embedded (so called germinal part). Cells of the glandular part are characterized by many cisternae of rough endoplasmic reticulum and Golgi bodies, often located close to the nucleus. Their nuclei are more or less rounded or slightly oval in shape, approximately twice as large as the nuclei of the somatic cells of the germinal part and located in the basal half of the cells (Fig. 2). Branching somatic cells forming a meshwork constitute the germinal part in which groups of sperm cells are embedded (Fig. 3). In contrast to the cells of the glandular part, the somatic cells of the germinal part are irregularly shaped and contain only a few cell organelles. Apically, in both somatic cell types there is a border of microvilli. Each sperm group consists of a few, probably four, mature sperm cells (Fig. 3). No spermatogenesis could be observed. The sperm cells float more or less distinctively in the narrow testicular lumen containing different kinds of proteinaceous secretions most likely produced by the glandular cells (Fig. 4). Towards the vasa deferentia and shortly before the testes open into the vas deferens, the epithelium flattens and no spermatozoa can be observed in the tissue. The sperm cells are rather simple, representing a roundish or slightly elongated cell body devoid of a flagellum, but provided with one, rarely two, flat extensions which fold onto the cell body (Fig. 8, 9, 10). In general, the following character-

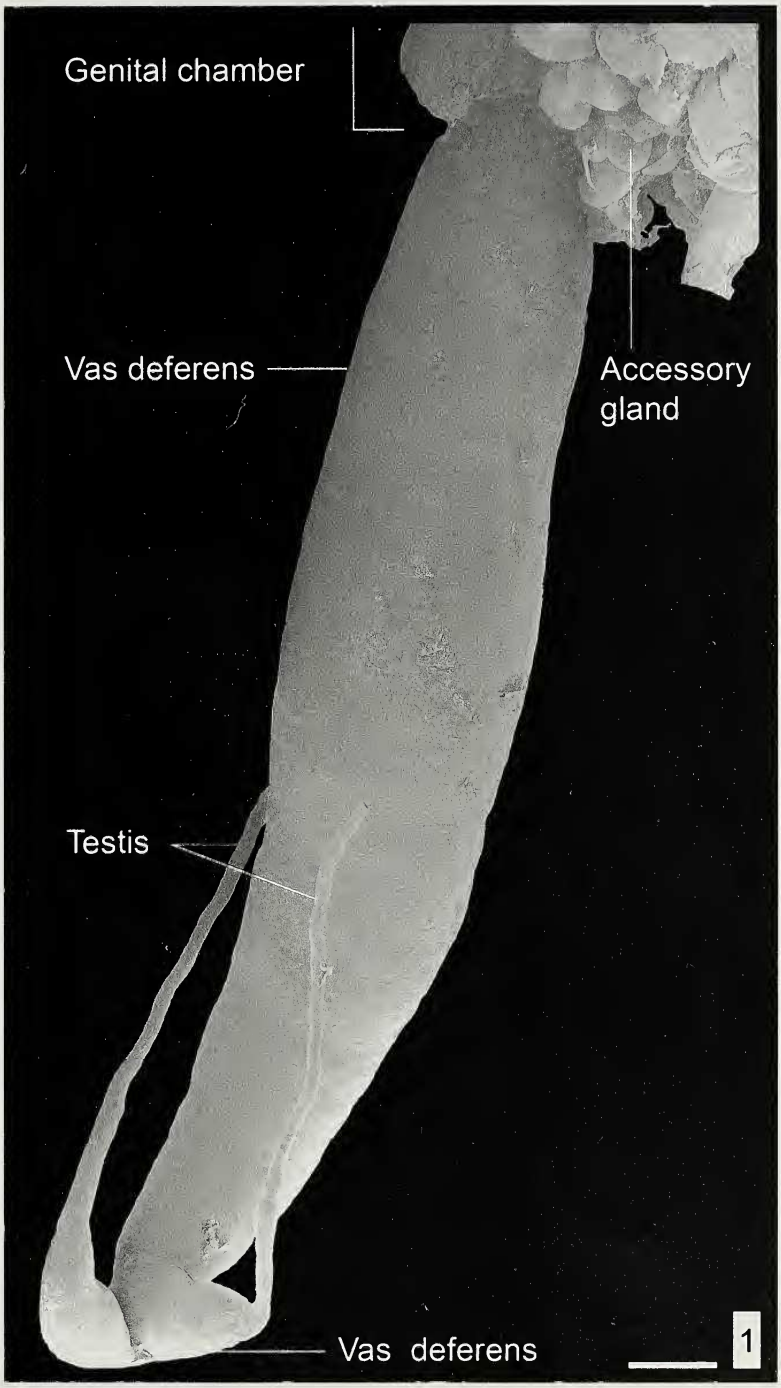
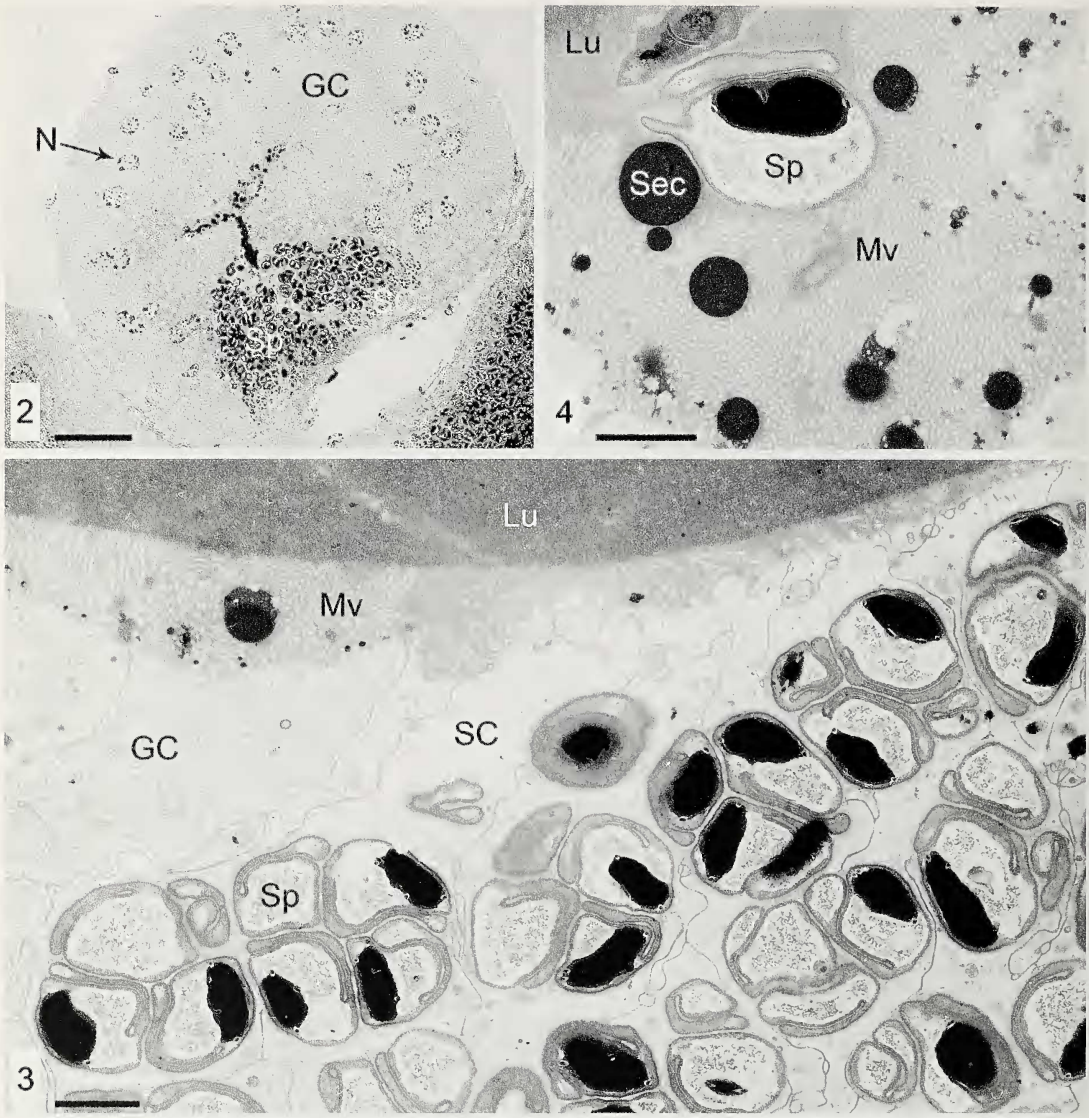


Figure 1.—Scanning electron micrograph of the left side of the male genital system of *Eremobates* sp. (genital chamber and testes are only partly shown; composed picture). Scale bar = 300 μm.

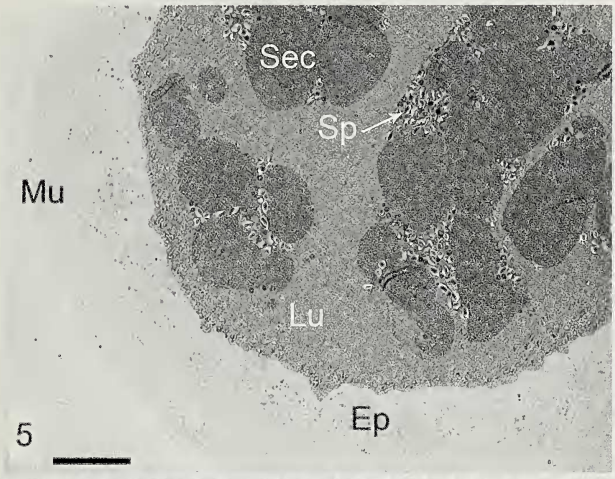
istic cell components can be distinguished in the mature spermatozoa: acrosomal complex, nucleus and cytoplasm including a more or less electron-lucent area. The acrosomal complex can be divided into an acrosomal vacuole, amorphous subacrosomal material and the acrosomal filament (perforatorium) starting from the amorphous subacrosomal material.

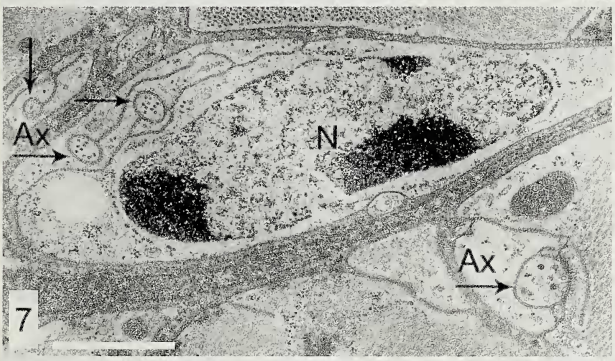


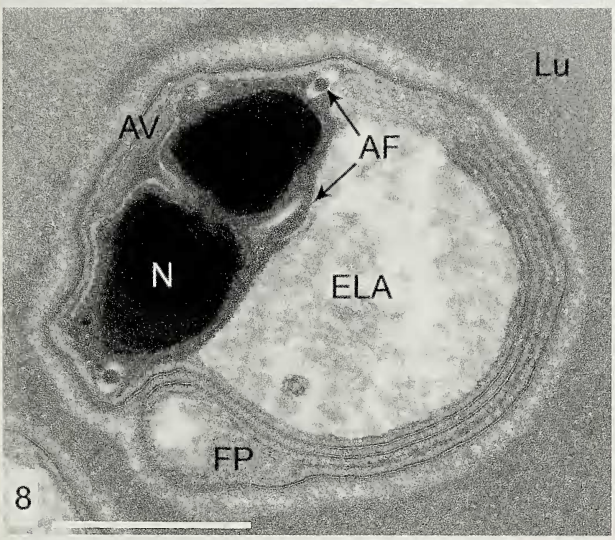
Figures 2–4.—Testis. 2. Light micrograph of the transversal section through the testis showing germinal and glandular part. Scale bar = 50 μm. 3. Groups of four spermatozoa embedded in somatic cells of the germinal layer. Left, glandular cells. Scale bar = 2 μm. 4. Sperm cell in the lumen of the testis surrounded by globules of secretions. Scale bar = 2 μm. Abbreviations: GC = glandular cell, Lu = lumen of the testis, Mv = microvilli, N = nucleus, SC = somatic cell, Sec = secretion, Sp = sperm cell.

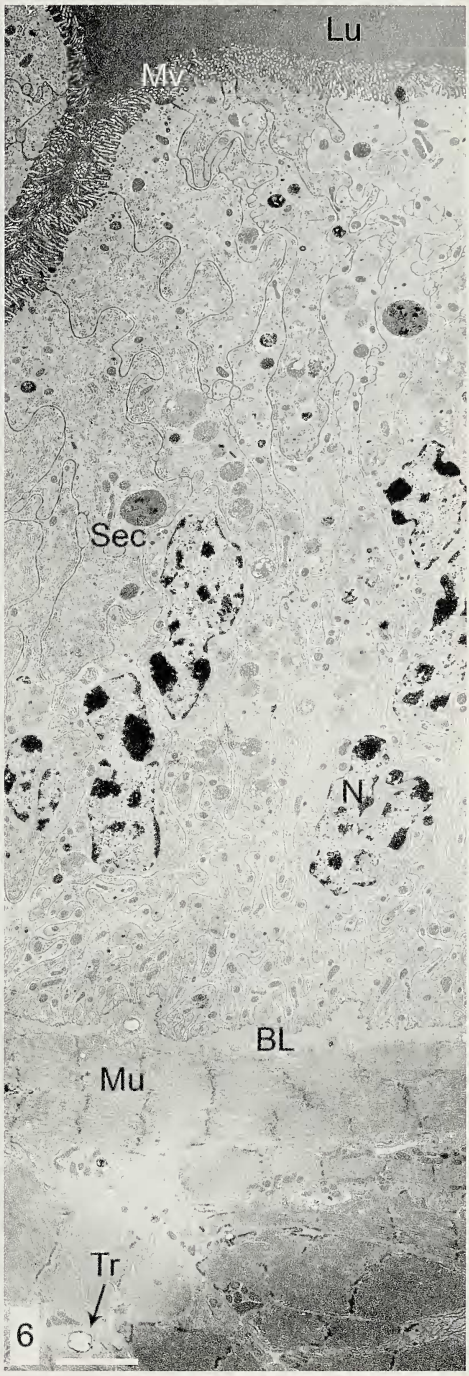
The relatively small acrosomal vacuole is attached to the electron-dense nucleus. The nucleus is penetrated and surrounded by the acrosomal filament (Figs. 8, 9). A conspicuous flat extension of the cell contains no organelles and slightly inflates towards its posterior end. The sperm cells show no tendency to form well ordered piles or globules either in the lumina of the testes or in the vasa deferentia.

Vas deferens: The epithelium of the vas deferens is underlain by a relatively thick outer cross-striated muscle layer interlaced with small tracheae (Figs. 5, 6). The epithelial cells are connected to the basal lamina via hemidesmosomes. The nuclei of the cells of the epithelium, containing considerable amounts of rough endoplasmic reticulum, are irregularly shaped. The wide lumen is filled with different kinds of secretions forming distinct

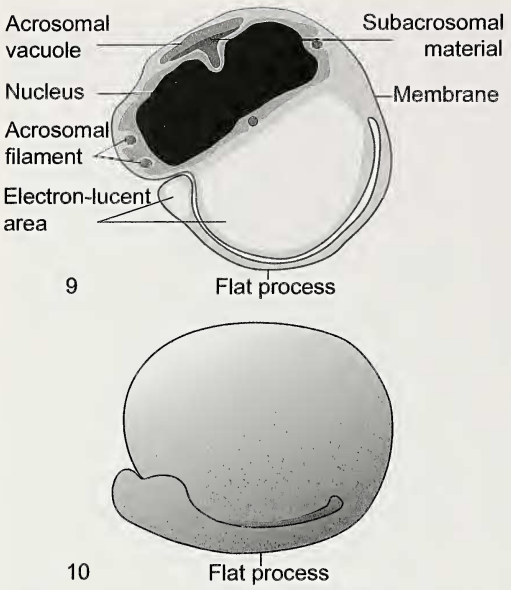








Figures 5–8.—Vas deferens. 5. Light micrograph of the small branch of the vas deferens. Scale bar = 50 μm. 6. Epithelium of the smaller branch of the vas deferens underlain by a muscle layer (composed picture). Scale bar = 4 μm. 7. Nerve fibres (indicated by arrows) within the muscle layer. Scale bar = 1 μm. 8. Single sperm cell in the lumen of the vas deferens. Scale bar = 1 μm. Abbreviations: AF = acrosomal filament, AV = acrosomal vacuole, Ax = axon, BL = basal lamina, ELA = electron-lucent area, Ep = epithelium, FP = flat process, Lu = lumen of the vas deferens, Mu = muscle, Mv = microvilli, N = nucleus, Sec = secretion, Sp = sperm cells, Tr = trachea.



Figures 9, 10.—Schematic drawings of a sperm cell. 9. Longitudinal section. 10. Three-dimensional reconstruction of the sperm body.

globules and mature sperm cells (Fig. 5). The muscle layer is innervated as indicated by the number of nerve fibres observed between the cells (Fig. 7).

Genital chamber: Several glandular pouches extend from the genital chamber and constitute the accessory glands. The glands are provided with an epithelium characterized by many rough endoplasmic cisternae, which are often inflated (Fig. 11). Secretory vesicles are only rarely observable. Apically, the cells bear microvilli (Fig. 12). The epithelium is underlain by thin muscle cells.

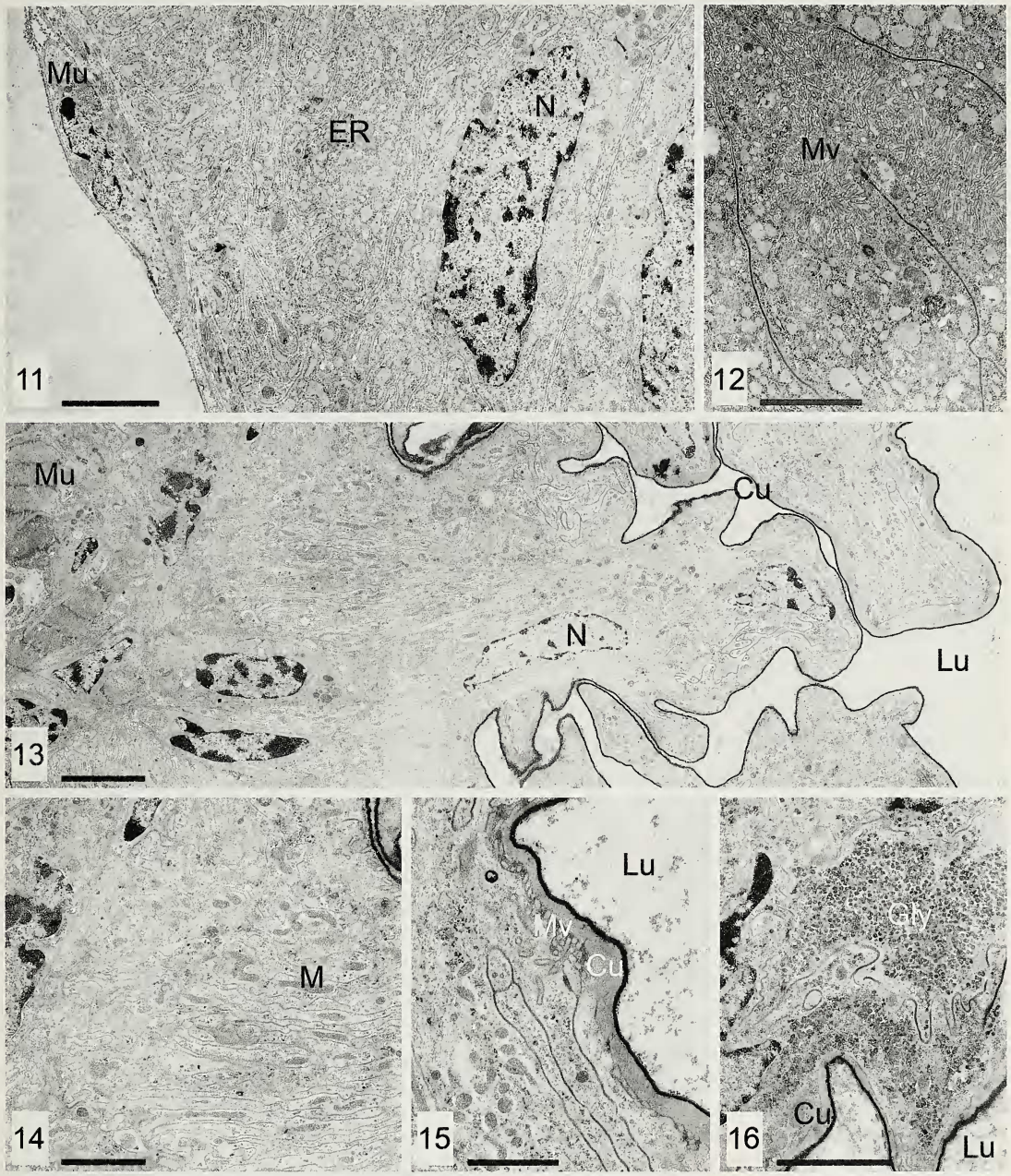
The genital chamber is directly connected to the genital opening located on the second opisthosomal segment. In certain regions the epithelium forms many finger-like processes extending into the lumen (Fig. 13). The epithelium of the genital chamber consists of a monolayer of cells which are characterized by basal membrane infoldings associated with mitochondria, thus forming a typical basal labyrinth (Fig. 14). Apically, the epithelium is provided with small microvilli over which a thin cuticle is located (Fig. 15). The cells sometimes contain extensive areas filled with glycogen (Fig. 16). A thick muscle layer, which is innervated, is located under the epithelium.

DISCUSSION

The two functionally different types of the epithelial cells of the testes in Solifugae have already been described by Alberti (1980) and Alberti & Peretti (2002). Our observations concerning the fine structure of the sperm cells agree with earlier results confirming the relatively simple ground pattern of sperm morphology in Solifugae. Nevertheless there are differences in the arrangement of the sperm. The observed spectrum in Solifugae covers highly ordered sperm cells in piles, both in the epithelium of the testes, in its lumen and in the lumen of the vas deferens of a karschiid species, groups of sperm that are less ordered and less compact in an ammotrechid representative and individual cells at least in the lumen of the vas deferens shown in an ammotrechid and the eremobatid species from Mexico studied here. Furthermore the sperm cells differ in shape and structural details. Some types of sperm cells exhibit membrane protuberances to various degrees whereas such structures cannot be observed in other representatives at all. However, it is still too early to apply these results to the systematics of Solifugae, since more species from other families need to be examined. The innervated musculature of the vasa deferentia is certainly involved in the transport of the sperm towards the genital opening and perhaps in releasing the sperm fluid.

Reports on sperm transfer differ. According to Heymons (1902), Cloudsley-Thompson (1961), Amitai et al. (1962) and Peretti & Willemart (unpub. data) sperm fluid is transferred semi-directly. A spermatophore or a sperm droplet is deposited by a male on the ground and subsequently picked up with his chelicerae and transferred to the genital orifice of the female. In contrast, Muma (1966, 1967) and Punzo (1998) reported a direct sperm transfer in the eremobatid solpugids *Eremobates durangonus* Roewer 1934, *E. palpisetulosus* Fichter 1941 and *E. nodularis* Muma 1951 from the genital orifice of a male to that one of the female.

The function of the accessory glands is speculative. One possibility is that they could take part in the formation of the sperm droplet. The extrusion of the secretion seems not to happen earlier than mating, since the lumina were almost empty in our specimens. A



Figures 11–16.—Genital chamber. 11. Periphery of an accessory gland (composed picture). Scale bar = 3 μm. 12. Cell apices of an accessory gland. Scale bar = 2 μm. 13. Epithelium overlain by a thin cuticle (composed picture). Scale bar = 5 μm. 14. Basal labyrinth characterized by membrane infoldings associated with mitochondria. Scale bar = 3 μm. 15. Cell apices of the epithelium with border of small microvilli. Scale bar = 2 μm. 16. Glycogen granules. Scale bar = 2 μm. Abbreviations: Cu = cuticle, ER = endoplasmic reticulum, Gly = glycogen granules, Lu = lumen, M = mitochondrion, Mu = muscle, Mv = microvilli, N = nucleus.

further source of secretion contributing to the formation of the sperm droplet could be the huge vasa deferentia and the glandular part of the testes. A similar function is known from actinotrichid mites (e.g., Alberti & Coons 1999).

Adults, in particular males, live only a short period of time after mating (Heymons 1902; Punzo 1998). Heymons (1902) in particular emphasized that the spermatophore (i.e. the drop containing sperm fluid) is reduced in size after several copulations. Junqua (1966) proposed that spermatogenesis occurs in subadult males prior to the adult molt which is supported by our ultrastructural investigations of adult males in which spermatogenesis was never detected (see also Alberti 1980; Alberti & Peretti 2002). Therefore it is reasonable to suggest that the testes and the vasa deferentia of an adult male serve only as storage sites for sperm cells until they are transferred during mating.

The apomorphic similarities in sperm cells and in the fundamental organization of the testicular tissue between Solifugae and actinotrichid mites have been pointed out by Alberti (1980) and Alberti & Peretti (2002). Although the Solifugae are commonly regarded as the sister–group of Pseudoscorpiones (together forming the taxon Haplocnemata, e.g. Weygoldt & Paulus 1979; Dunlop 2000), there are tremendous differences in sperm morphology. Pseudoscorpiones possess complex coiled-flagellate spermatozoa (e.g., Werner & Bawa 1988; Dallai & Callaini 1990; Alberti 2000). Thus, comparative spermatology does not support a close relationship between these two animal groups. However, the assumption that the Acari represent a monophylum may be questioned (Alberti 2000; Alberti & Peretti 2002). It may be argued that the differences in the mode of sperm transfer, indirect spermatophore transfer in Pseudoscorpiones and direct or semi-direct in Solifugae, may consequently be reflected in different sperm types. These differences may not necessarily contradict a sister-group relationship between Pseudoscorpiones and Solifugae. However, it can be shown in other arachnid taxa with comparable sperm transfer, e. g., Araneae or Ricinulei, that sperm morphology is not necessarily modified in the same manner as in Solifugae or actinotrichid mites (Alberti 2000). Furthermore, actinotrichid mites show three kinds of sperm transfer: indirect spermatophore transfer, direct spermatophore transfer using gonopods and direct insemination via a penis, all possessing simple aflagellate spermatozoa. Evidently there is no simple correlation between sperm structure and mode of sperm transfer (Weygoldt 1990, Alberti & Peretti 2002). The similarity in the testis histology in the Solifugae and actinotrichid mites is remarkable. If the Solifugae are closely related to the Pseudoscorpiones (as suggested above), the similarity of the testis histology and the aflagellate sperm must be seen as homoplastic. Another interesting aspect is the occurrence of a transport epithelium in the genital chamber, characterized by conspicuous infoldings of membranes associated with numerous mitochondria. Such a distinct epithelium is also present in the genital papillae of actinotrichid mites (Alberti & Coons 1999), but evaluation of this character in terms of phylogenetic systematics requires further investigations on a broader range of taxa.

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